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MECHANISMS OF DEVELOPMENT

Formation of the zebrafish midbrain–hindbrain boundary constriction requires laminin-dependent basal constriction

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ABSTRACT

The midbrain–hindbrain boundary (MHB) is a highly conserved fold in the vertebrate embryonic brain. We have termed the deepest point of this fold the MHB constriction (MHBC) and have begun to define the mechanisms by which it develops. In the zebrafish, the MHBC is formed soon after neural tube closure, concomitant with inflation of the brain ventricles. The MHBC is unusual, as it forms by bending the basal side of the neuroepithelium. At single cell resolution, we show that zebrafish MHBC formation involves two steps. The first is a shortening of MHB cells to approximately 75% of the length of surrounding cells. The second is basal constriction, and apical expansion, of a small group of cells that contribute to the MHBC. In the absence of inflated brain ventricles, basal constriction still occurs, indicating that the MHBC is not formed as a passive consequence of ventricle inflation. In laminin mutants, basal constriction does not occur, indicating an active role for the basement membrane in this process. Apical expansion also fails to occur in laminin mutants, suggesting that apical expansion may be dependent on basal constriction. This study demonstrates laminin-dependent basal constriction as a previously undescribed molecular mechanism for brain morphogenesis.

1. Introduction

During development of the vertebrate brain, the neural tube assumes a complex structure that includes formation of the brain ventricles and the appearance of conserved folds and bends. These folds and bends delineate functional units of the brain and are likely to shape the brain such that it can pack into the skull. The midbrain–hindbrain boundary (MHB) is the site of one of the earliest bends in the developing brain. In the embryo, the MHB functions as an embryonic organizing center (Brand et al., 1996; Joyner, 1996; Puelles and Martinez-de-la-Torre, 1987; Sato et al., 2004) and later becomes the cerebellum and part of the tectum (Louvi et al., 2003).

We have called the deepest point in the MHB the "midbrain–hindbrain boundary constriction" (MHBC). In the present study, we ask what processes are necessary for MHBC morphogenesis, using the zebrafish as a model. In the

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zebrafish, the MHBC forms between 17 and 24 hours post fertilization (hpf), concomitant with formation of the brain ventricles. At this stage of development, the neuroepithelium is a pseudostratified-columnar epithelium where apical cell surfaces face the brain ventricle lumen, and basal cell surfaces, on the outside of the tube, abut the basement membrane.

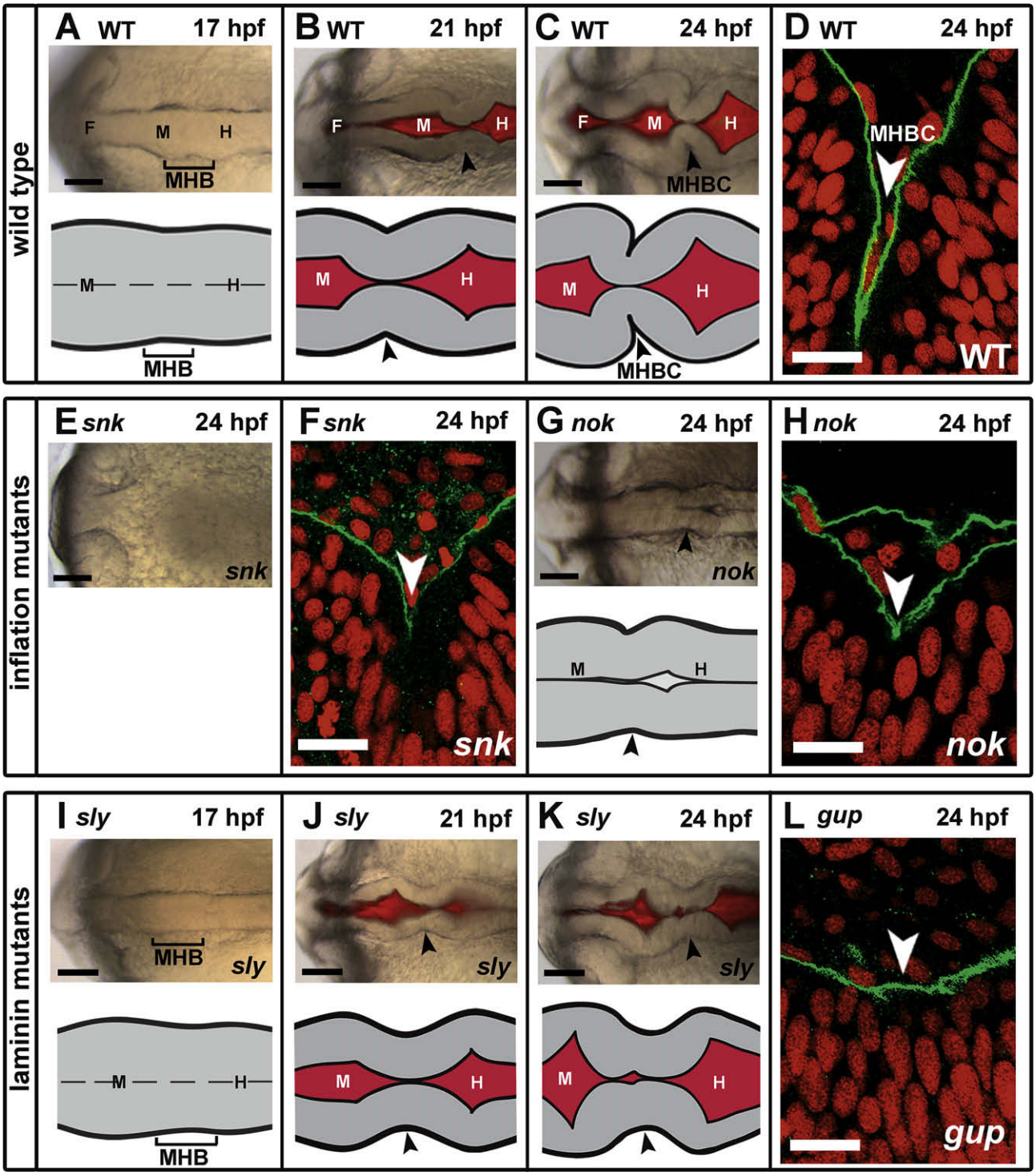

Fig. 1 – Zebrafish MHB morphogenesis occurs between 17 and 24 hpf, and requires laminin but not ventricle inflation. (A–C) Brightfield and fluorescent images and schematics of wild type (WT) MHBC formation. (D) WT embryo at 24 hpf was stained with Laminin 1 antibody (green); nuclei were stained with propidium iodide (red). Laminin lines the basal surface of the neuroepithelium. (E) Brightfield image of *snk*, a ventricle inflation mutant, at 24 hpf. (F) *snk* embryo at 24 hpf stained as in (D). (G) Brightfield image and schematic of *nok*, a ventricle inflation mutant, at 24 hpf. (H) *nok* embryo at 24 hpf stained as in as (D). (I–K) Brightfield and fluorescent images and schematics of MHBC formation in the laminin mutant, *sly*. (L) *gup* embryo at 24 hpf stained as in (D). Arrowheads indicate MHB at 21 hpf and MHBC at 24 hpf. F, forebrain; M, midbrain; H, hindbrain. Scale bars: A–C, E, G, I–K = 100 μM, D, F, H, L = 6 μM.